High-resolution microcontact printing and transfer of massive arrays of microorganisms on planar and compartmentalized nanoporous aluminium oxide†

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Handling microorganisms in high throughput and their deployment into miniaturized platforms presents significant challenges. Contact printing can be used to create dense arrays of viable microorganisms. Such "living arrays", potentially with multiple identical replicates, are useful in the selection of improved industrial microorganisms, screening antimicrobials, clinical diagnostics, strain storage, and for research into microbial genetics. A high throughput method to print microorganisms at high density was devised, employing a microscope and a stamp with a massive array of PDMS pins. Viable bacteria (*Lactobacillus plantarum*, *Esherichia coli*), yeast (*Candida albicans*) and fungal spores (*Aspergillus fumigatus*) were deposited onto porous aluminium oxide (PAO) using arrays of pins with areas from 5 × 5 to 20 × 20 μm. Printing onto PAO with up to 8100 pins of 20 × 20 μm area with 3 replicates was achieved. Printing with up to 200 pins onto PAO culture chips (divided into 40 × 40 μm culture areas) allowed inoculation followed by effective segregation of microcolonies during outgrowth. Additionally, it was possible to print mixtures of *C. albicans* and spores of *A. fumigatus* with a degree of selectivity by capture onto a chemically modified PAO surface. High resolution printing of microorganisms within segregated compartments and on functionalized PAO surfaces has significant advantages over what is possible on semi-solid surfaces such as agar.

1. Introduction

Since the 19th century microbiologists have been handling microorganisms with simple but effective manual tools which have not changed greatly since their invention. Typically, sterile toothpicks or a wire loop are used to isolate a sample of microbial cells which can be purified to homogeneity by streak-plating to a colony forming unit (CFU). A simple but significant advance in the 1950s was to hand print arrays of hundreds of microbial colonies (as replicates across several agar plates) using a sterile velvet pad or array of pins.[1] Replica plating facilitated screening of microbial populations for individual lineages with a desired genetic trait. By means of such screenings, microbial genetics and biochemistry and the improvement of industrial strains of microorganisms were advanced. Such screenings are often a painstaking and manual process; typically hundreds of strains are replicated at a time whilst the frequency of desired mutants is considerably below 1 in 10^6.

Developments in how microorganisms are studied (*e.g.* systems biology and an increased interest in the still-uncultured majority of microorganisms), the need for engineered microorganisms with improved characteristics (*e.g.* metabolic engineering, synthetic biology) and the continuing threat of microbial pathogens (*e.g.* the spread of acquired antibiotic resistance) are drivers for improved methods for handling and growing microorganisms. Microbial culture is not yet a highly automated discipline, and attempts to introduce automation have often resulted in large machines that are not economic for routine microbiology laboratories. With the exception of bulk industrial processes, most microbiology is about the detection and subsequent analysis of microorganisms. The length of a typical microorganism is from 0.5 to 10 μm and only small numbers are needed for many analytical processes. Therefore, microbial culture may often be scaled down with benefits in cost and efficiency, and reductions in waste and biohazard. Additionally, with MEMS culture devices it may be possible to run current assays with unprecedented throughput or with the integration of multiple steps; for example microbial culture with molecular assays.[2,3] Part of the challenge of miniaturizing and automating microbiology is how to handle (move, purify, inoculate) microorganisms, especially since microbial cells are highly heterogenous and diverse in their physical and chemical properties.

Microorganisms can be moved or directed by many means; they may be patterned (recruited to surfaces *via* affinity chemistry), moved by electrical forces (*e.g.* dielectrophoresis or when

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conjugated to particles),[4] directed by liquid flow, be induced to move themselves (motility directed by physical constraints or a sensory process) or be physically stamped or otherwise printed. Exceptionally precise contact printing methods have been developed in recent years.[5] Printing is highly relevant for high throughput microbiology; both contact printing and non-contact printing of arrays of microorganisms have been demonstrated in recent years. Printing microbes has other applications, such as building structured communities for research or for integrating cells into bioMEMS devices.[2,3,6,7] Robotic systems exist (*e.g.* Genetix Qbot) that can perform multipin inoculations but these currently print to a limited density: current commercial systems operate at a pitch on the millimeter scale. Piezoelectric and other non-contact printers (including widely available and low-cost bubble-jet printers) have also been adapted to microorganisms. Such printers can be used to inoculate growth medium (such as a nutrient agar plate). Despite transitory heating effects and shear forces during printing, viability of printed bacteria is high although some cellular structures such as capsules may be damaged.[8,9] However, separation between printed colonies is only moderate, with pitches of hundreds of microns to millimeters being typical. There are also issues of accuracy due to droplet deflection and the probability a droplet will contain a single CFU. Microcontact printing, *e.g.* using elastomer stamps, offers an alternative. Microcontact printing of bacteria with agarose pins has allowed repeated inoculations with pitches from a few millimeters and allow "regeneration of the ink" by microbial growth on the pins.[10] Printing with much finer polydimethoxysiloxane (PDMS) pins (as small as 6 μm across) can be used to inoculate small numbers of viable bacteria, effectively allowing a microcontact printer to act as a wire loop, *i.e.* as a tool to purify strains to a single CFU.[7]

One limitation of printing methods for microorganisms is the target; typically this is the complex polysaccharide agar or agarose, the matrix most commonly used to gel the nutrients that support growth in traditional microbiology. There are problems here: the agar surface is not usually divided into separate growth areas, which limits the density of discrete inoculations, particularly after outgrowth. Additionally, on the micron scale agar can have quite significant surface imperfections. High percentage agars (3 to 4% w/v) appear most useful in the highest resolution micro-printing studies[7] but these are not the best formulations for microbial growth, which is more usually performed on lower percentage agars, most commonly from 0.7 to 1.8% (w/v). There are also limitations to the extent to which agar can be chemically modified or functionalized. Additionally, agar may not be the most convenient matrix to deploy in future miniaturized or high throughput microbial culture formats. Alternative targets to agar include printing on planar solid surfaces but these are not suitable for the growth of microorganisms in most cases. Other printable surfaces can act as a matrix for microbial growth, including nylon membranes, allowing nutrient delivery from beneath through the pores.[2] Porous aluminium oxide (PAO) is not toxic to bacteria or fungi and allows microbial growth on the surface with nutrients supplied from beneath.[11] PAO presents a flat surface, and can be chemically modified or physically subdivided into separate culture areas as small as 7 μm across, creating massively parallel culture arrays, potentially with millions of strains.[12] In this article we report on high-resolution microcontact printing and transfer of massive arrays of microorganisms on planar and compartmentalized porous aluminium oxide.

2. Materials and methods

2.1 Design and construction of printing set up

The printing set-up was built around an Olympus BX 41 fluorescence microscope equipped with a Kappa CCD camera and a crosshair eyepiece. An automated XY table with motorized Z-axis (Marzhauser, DE) controlled by a Corvus controller and a rotation facility were used to allow precise positioning of the chip relative to the objective lens (Fig. 1). The optics of the microscope were modified by inserting an additional convex lens enabling a shift of focus between the stamp and target. An adaptor (Fig. 1 and ESI Fig. 1†) was made, to connect the stamp to the 10× microscope objective by vacuum clamping. The adaptor itself was clamped to the objective. A hole in the bottom of the adaptor, together with a convex lens, allowed observation of either the stamp or the surface to be printed.

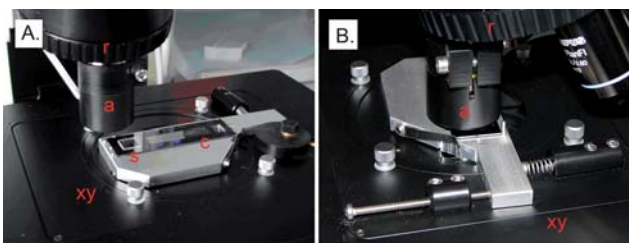

Fig. 1 Photograph of microscope stage during printing setup. (A) View of setup. The adapter (a) holds the stamp in the focal plane of a ×10 microscope objective. The stamp can be rotated in or out using a standard rotating carousel on the microscope (r). On the XY-table (xy) is clamped a chip holder with the 10 × 10 mm transparent stamp (s) and an 8 × 36 mm area for a chip or strip of PAO (c). Movement of the stage (X, Y and rotation) allows positioning of the culture chip relative to the objective lens. (B) View during printing, with stamp positioned with the pin tips 10 μm above the chip (both hidden from view by adaptor).

Table 1 Dimensions of replicator pads (stamps) manufactured and used. Pin height and pitch are in μm

Pin area	Height	Pitch	Array	Total no. pins
5 × 5	14	10	100 × 100	10 000
5 × 5	14	15	100 × 100	10 000
5 × 5	14	15	600 × 600	360 000
5 × 5	14	15	200 × 200	40 000
10 × 10	14	var[a]	50 × 50	2500
10 × 10	15	35	50 × 50	2500
20 × 20	20	70	10 × 10	100
20 × 20	20	70	50 × 50	2500
20 × 20	20	70	90 × 90	8100

[a] Pitch was variable. In the X-axis the left edge of the first pin (top left) was 6 μm from the left edge of the second (1 μm gap, 6 μm pitch), the third pin was 7 μm from the second with the pitch and the gap increased by 1 μm with each successive pin. Similarly, the spacing of pins was incremented within the Y-axis. Therefore, the pins had maximum density in the top left corner of the replicator pad and were most widely spaced at the bottom right.

PDMS stamps were fabricated with a printing area that was square in cross-section, ranging from 5 × 5 to 20 × 20 μm with pin lengths of up to 20 μm and pitches from 6 to 70 μm (Table 1 and Supplementary Methods†). This setup allowed precise positioning of the stamp during printing, distortion from focusing through the stamp when viewing the target beneath was minimal. Loading the stamp with microorganisms was by alignment of the PDMS stamp with the target (PAO coated with microorganisms) then dropping the stamp from a height of 5 μm. Subsequent printing actions were by picking up the stamp, aligning it with the target (PAO or a compartmentalized PAO culture chip) and dropping it. A detailed protocol for loading and printing can be found in the Supplementary Methods†. Printing actions took approximately 30 s (loading), 1 min (printing on unstructured PAO) and 2.5 min (printing in compartmentalized PAO).

2.2 PAO handling, PAO chemistry and PAO culture chips

Strips of PAO (8 × 36 mm area, 60 μm thick, 0.2 μm pores, 40% porosity) were sterilized and moved on strips of sterile Parafilm as described previously.[12] Culture chips (40 × 40 μm growth area, 30 μm wide walls, 10 μm high) were created by laminating PAO strips with Ordyl SY300 film[13] followed by reactive ion etching, with a silicon shadow mask as the pattern determining device. A platinum coating (20 nm thick) was applied to block excessive autofluorescence from the Ordyl film. Fabrication of a silicon holder for the support of PAO during printing was also as previously reported.[12] Poly-L-lysine (PLL) treated PAO was prepared as previously described; heparin treatment was similar.[14] Hydrophobic PAO was created by coating with HDMS (hexamethyldisilazane, Thermo Scientific, USA) or EGC1700 (3M, USA) for 10 min at room temperature then baking at 200 °C for 10 min.

2.3 Microbiology

Cultures of the Gram-positive bacterium *Lactobacillus plantarum* WCFS1, the Gram-negative bacterium *Eschericia coli* XL2 Blue, the yeast *Candida albicans* JBZ32 and spores of the filamentous fungus *Aspergillus fumigatus* JBZ1 were used as the "ink" in printing experiments. Where necessary, these microorganisms were either stained with fluorogenic dyes (*L. plantarum*, *C. albicans*, *A. fumigatus*), or had quantum dots conjugated to the cell wall (*L. plantarum*) or were engineered to express green fluorescent protein (*E. coli*). These measures facilitated detection and allowed assessment of the printing efficiency by fluorescence microscopy. Details of the culture, labelling and handling of microorganisms are given in the Supplementary Methods.†

2.4 Imaging and analysis

TIFF format images captured by a Kappa CCD camera were analyzed using the Java-based imaging software package ImageJ version v1.37.[15] ImageJ cell counting macros were used to quantify numbers of printed bacteria and fungi. Printing in limited fields of view was verified by fluorescence microscopy. Printing in low-magnification fields of view was scored as successful if the fluorescence level was >3 S.D. above background. Statistical calculations were performed in Microsoft Excel or using the Vassar Statistics web server.[16]

3. Results and discussion

3.1 Loading pins with microorganisms

Initial printing experiments of microorganisms onto PAO used the bacterium *Lactobacillus plantarum* labeled with quantum dots (QDOTS) attached to the cell wall by a wheat germ agglutinin linker. A viable count on MRS agar plates indicated that >96% of bacteria remained viable after conjugation. The pins

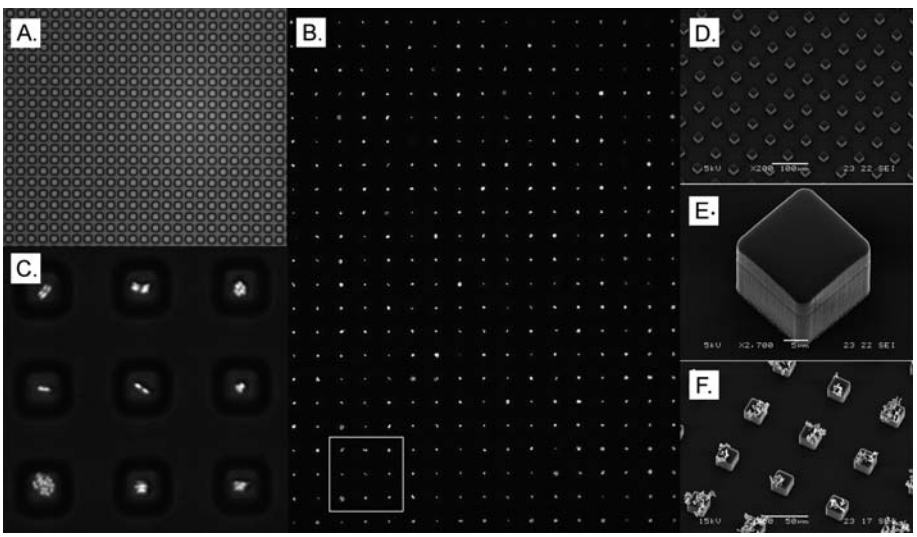

Fig. 2 Examples of replicator stamps viewed by microscopy. (A) Part of an array of 5 × 5 μm pins (10 μm pitch) viewed by light microscopy. (B) Example of 10 × 10 μm pins (35 μm pitch) after loading with *L. plantarum* labeled with the fluorogenic dye Syto 9 and imaging by fluorescence microscopy. Printing in this case was designed to pick up a small number of cells to allow individual bacteria to be seen; printing experiments generally used a heavier loading of the pins (>50-fold more). (C) Detail from panel B (within area defined by the white square) merged with a transmission light microscopy image of the pins. (D to F) Replicator stamps (20 × 20 μm pins) viewed by scanning electron microscopy. (D) Overview. (E) Detail of a single pin. (F) Pins loaded with spores of *A. fumigatus*.

were loaded by releasing the stamp to drop onto a paste of bacteria (>10^{12}/ml) on PAO held flat on a microscope-footprint holder. Subsequent examination of recovered stamps (5 × 5, 10 × 10 or 20 × 20 μm printing area) by microscopy indicated that, for all formats, >99.95% of the PDMS stamps was not damaged by dropping. The high efficiency and consistency of loading by dropping the stamp was in contrast to delivery of the stamp by the Z-axis motor. Despite decelerating the stamp when closing with the target PAO it was not possible to achieve >71% loading with the pins undamaged. Damage or partial loading occurred in this scenario, with a strong bias to one edge of the array of stamps, *i.e.* the pins were not perfectly parallel to the PAO surface when descending and therefore either failed to contact or were damaged during contact. Dropping the 100 mg PDMS stamp a few microns allowed correction of any such variation with minimal impact stress. Therefore, the same dropping technique was also used for subsequent printing actions.

After recovery of the stamp, pins were examined directly for the presence of microorganisms (Fig. 2). With the standard loading with cells >99.9% of pins of all configurations (n = 3000) picked up cells of *L. plantarum* or *E. coli.* Examination of >200 stamps (20 × 20 μm pin area) suggested >200 bacteria per pin were picked up during loading. Eliminating the Ficoll and glycerol (both present at 5% w/v) from the bacterial paste decreased the efficiency of loading >5-fold. These additives were necessary to prevent desiccation of the sample during printing. Loading pins with cells of the pathogenic yeast *C. albicans* and spores of the filamentous fungi *A. fumigatus* was also possible by the same method. Primarily due to larger size the pastes of these organisms contained approximately 10-fold fewer organisms (*ca.* 10^{11}/ml) than the *L. plantarum* preparations. Examination of pins by light microscopy suggested that >25 cells or spores per 20 × 20 μm pin were being loaded. Eliminating the Ficoll and glycerol from the loading mix decreased the efficiency 2.6-fold for *C. albicans* and 1.4-fold for *A. fumigatus*. Loading of cells of all types appeared proportionately less for pins of smaller areas (5 × 5 and 10 × 10 μm).

3.2 Printing on unmodified PAO

After loading with bacteria the stamps were dropped onto the PAO surface from a height of <5 μm to deliver bacteria to the surface. Printing *L. plantarum* onto PAO with a stamp of incremental pitch (increasing the spacing between 10 × 10 μm pins by 1 μm from 2 μm) indicated that the minimum print distance was 17 μm pitch (7 μm spacing). When pins were fabricated closer together they tended to aggregate in 2 × 2 groupings and so could not reliably print separately. Printing success was scored using QDOT conjugated bacteria when the fluorescence of the target area was >3 S.D. above background. Printing and viability was confirmed by the outgrowth of microcolonies. With constant pitch stamps extremely large arrays of multipin inoculations were made (Fig. 3). Using a stamp and scoring 1000 pins (5 × 5 μm cross-section) a success rate of >93% could be obtained. With 1000 (10 × 10 μm or 20 × 20 μm) pins a success rate of >96% could be obtained. Subsequent culture indicated that inoculations were of viable bacteria and fungi as indicated by outgrowth after 1 h for *E. coli*, 2–4 h (*L. plantarum*, *C. albicans*) or germination after 8 h (*A. fumigatus*). As with loading, dropping the stamp was not damaging.

With a single loading, four printings were performed in different areas of a PAO strip (Fig. 4). Arrays of 20 × 20 μm pins

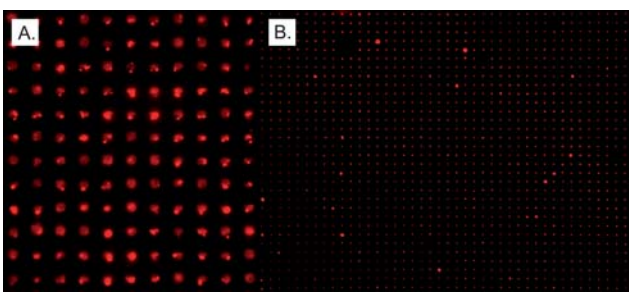

Fig. 3 *L. plantarum* cells printed onto PAO and visualized by fluorescence microscopy. (A) Printing with pins of 20 × 20 μm cross-section and a pitch of 70 μm. Each pin has printed from 10 to 50 cells of *L. plantarum* labeled with QDOTS. (B) Overview of part of a large printing experiment with pins with a cross-section of 10 × 10 μm and 35 μm pitch. Up to 20 cells of *L. plantarum* were printed per pin.

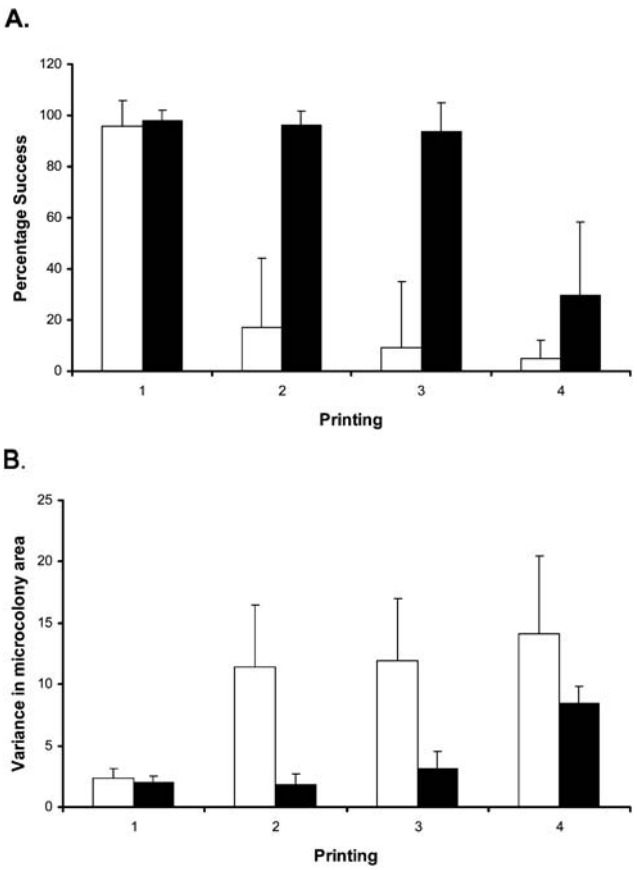

Fig. 4 Efficiency of printing bacteria repeatedly from a single loading. (A) Printing efficiency of 10 × 10 μm area pins (white shading) and 20 × 20 μm pins (black shading) ± S.D. from the mean (3 replicates). *L. plantarum* cells labeled with QDOTS were printed repeatedly after loading once then scored for % pins printing on PAO. N > 1000 pins per replicate. (B) Same experiment as (A), showing the increase in variance with successive printings, calculated from the area of the printed microcolonies. 10 × 10 μm pins (white shading) and 20 × 20 μm pins (black shading) ± S.D.

(90 × 90 pins; 8,100 pins) proved optimal, for these three successive printings could be performed with high efficiency, with increasing variance but only a slight decrease in the percentage of successful inoculation. With 10 × 10 μm pins only the first printing was high quality (low variance, high efficiency). Therefore, we conclude that for creation of multiple replicates 20 μm pins are required. However, for printing to a single CFU with a minimal number of steps, *i.e.* use as a "wire loop" to purify strains, smaller pins (5 × 5 and 10 × 10 μm print area) combined with lower levels of loading with bacteria were appropriate. Two replicates with 5 × 5 μm pins were sufficient to purify *L. plantarum* to single CFUs with >80 μm separation between most (>95%) of inoculations.

3.3 Printing on functionalized PAO

A desirable property of a microbial printing device is to make selective transfers of microorganisms. This would facilitate enrichments and culture-based assays that incorporate information as to the surface properties, for example the existence of conditionally expressed pathogenicity determinants. Therefore, in order to test the effect of simple alterations in surface chemistry, PAO strips were treated with poly-L-lysine (positively charged), HDMS (hexamethyldisilazane) or EGC1700 (both hydrophobic) or heparin (negatively charged) or left unmodified. *C. albicans* and *A. fumigatus* spores were chosen for these experiments and they are both fungi and in these morphotypes are of similar sizes (3 to 5 μm diameter particles approximating to a sphere) but with contrasting surface properties. Ungerminated *A. fumigatus* spores are exceptionally hydrophobic for a microorganism.[17] *A. fumigatus* spores were surface-labeled with the protein stain NanoOrange (Supplementary Fig. 2) and *C. albicans* with the fluorogenic dye Syto 9, allowing printing efficiency to be independently assessed by fluorescence microscopy. The ratio of adhesion (*Aspergillus*/*Candida*) to the stamp after loading with this mixture was on average (n = 3 experiments, 200 pins measured/experiment) 0.81, a slight enrichment for *C. albicans* compared to the initial mixture (Table 2). Printing (3 replicates, 200 pins analysed/experiment) showed that the two hydrophobic coatings (HDMS, EGC1700) increased the efficiency of printing spores (EGC1700 5.4-fold, HDMS 6.1-fold) compared to unmodified PAO. Poly-L-lysine enhanced printing of both organisms, but was more effective for *C. albicans*. This differential printing efficiency indicated that by modifying the target of the PAO, organism-specific selectivity could be obtained. This suggests that a degree of selectivity is possible and opens the way to the use of more highly specific capture agents. For example, capture agents such as antibodies and carbohydrates can be immobilized in an active form on PAO.[14,18] This may open up applications in selective purification of microorganisms and high throughput screening based on surface properties such as bacterial display libraries or screening for microorganisms that bind to a molecular scaffold.

Table 2 Printing fungi on modified PAO with different surface chemistries

	Modification to PAO				
Organism(s) printed	None	HDMS[a]	EGC1700	PLL[b]	Heparin
C. albicans	1	1.3	0.9	6.1	1.3
A. fumigatus spores[c]	1	6.1	5.4	2.0	0.8
Relative enrichment of *A. fumigatus*[d]	1	5.4	3.61	0.41	0.32

[a] Hexamethyldisilazane. [b] Poly-L-lysine. [c] Printing efficiency of cells of *C. albicans* and spores of *A. fumigatus* relative to the printing efficiency on unmodified PAO. [d] Printing efficiency of a mixture of the two; the enrichment is of *A. fumigatus* relative to *C. albicans* (1 : 1 ratio) relative to the printing of the mixture on unmodified PAO.

3.4 Printing on compartmentalized PAO

High density printing on an unstructured planar surface leads to a serious problem with microbial containment. After minimal outgrowth cross-contamination between initially distinct microcolonies is inevitable and makes screening of growing microorganisms difficult. Indeed, after printing with the 20 × 20 μm pins culture for only two rounds of division (typically <4 h for *L. plantarum* and <2 h for *E. coli*) led to a near-confluent monolayer of cells with little segregation or resolution. Therefore, printing for longer-term growth and handling was performed in culture chips with 40 × 40 μm culture areas on an 8 × 36 mm PAO base (42 000 culture areas/chip). The growth compartments were defined and segregated with 30 μm wide, 10 μm high walls of acrylic plastic covered with a 20 nm layer of platinum to reduce autofluorescence. The chips used were sterilized and handled as described previously.[12] In order to print the chips with *L. plantarum* a precise alignment of pins with growth compartments was required. This was done by imaging one corner of the transparent stamp and the culture chip beneath by microscopy (Fig. 5). Alignment was facilitated by an additional lens that allowed the focal plane to be switched between the stamp and the chip below. The chip was moved relative to the stamp using the XY-table so that the stamps were placed <5 μm above compartments then dropped to load with bacteria or print as described for unstructured PAO. Printing within these wells was possible, using stamps inoculated with cells from another region of the

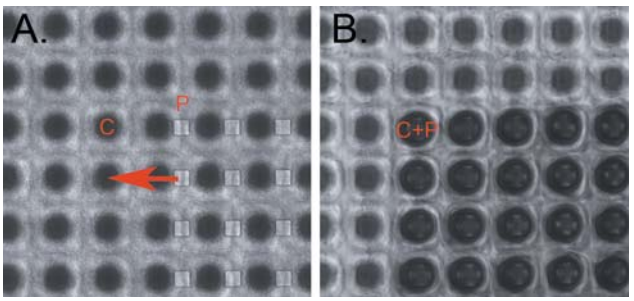

Fig. 5 Example of fields of view (by microscopy from above) seen during alignment and printing in compartmentalized PAO culture chips. (A) View of one corner of an array of stamps and a portion of the target culture chip. The focal plane is on the transparent pins (P); the 40 × 40 μm compartments (C) of the PAO chip are tens of microns below the stamp and therefore the chip is slightly out of focus. The chip is viewed through the transparent stamp. The stamp is not yet aligned in the X-axis so the pins are above the walls of the compartments. (B) After movement of the stamp 35 μm to the right the pins are now aligned with the PAO compartments and has been dropped so that the pins contact the PAO base of the compartment (*e.g.* C + P).

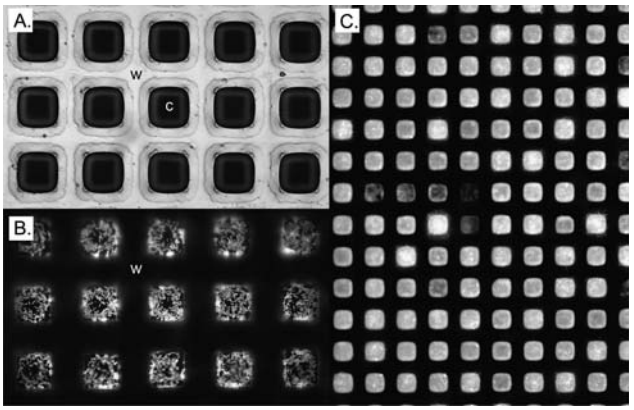

Fig. 6 Printing cells of the bacterium *L. plantarum* in PAO culture chips with 40 × 40 μm growth compartments. (A) Transmission light microscopy of section of an unprinted chip with compartments marked C, subdividing walls annotated W. (B) Fluorescence light microscopy of a section of a printed chip after printing, culturing for 4 h then staining the cells of *L. plantarum* in the compartments with the fluorogenic dye Syto 9. Bacteria appear white against a non-fluorescent background. (C) As (B), but overview of a larger printed area (>100 compartments) after 8 h incubation, with all compartments inoculated and most filled with growing bacteria but without cross-contamination between different areas.

same chip. Printing of QDOT conjugated *L. plantarum* was >93% successful with 20 × 20 arrays of 20 × 20 μm pins (n = 3). *L. plantarum* inoculated into these compartments were viable. The compartmentalized nature of the PAO surface permitted effective segregation for up to eight rounds of division (Fig. 6). The primary limitation was alignment precision; with more precise positioning of the stamp relative to the grid of the culture chips larger arrays should be printable.

4. Conclusions

Handling microorganisms poses a significant challenge: they are physically and chemically diverse particles which may pose a health hazard or spoilage problem if improperly deployed. Here we have developed a novel printing system, one that uses minimally modified features of a microscope to deliver arrays of thousands of viable microorganisms at high density, more so than other methods. Additionally, by printing multiple times, the method may be used to purify to single colony units within a small area, potentially with a degree of selectivity. Critical to the printing process was that the replicator was dropped for the last few microns, ensuring a "soft landing". This allowed extremely large numbers of samples to be delivered compared to other contact printing methods. Additionally, we chose to print on a nanoporous ceramic surface (PAO) rather than agar. PAO has the advantages of greater flatness and consistency and avoids the use of high agar concentrations. Arrays of microorganisms on PAO can be readily subjected to changes in their environment permitting sophisticated nutritional shifts and more complex assays than are possible on agar. Furthermore, the surface chemistry of PAO can be modified in ways that agar cannot. For example, chemical modification leading to an increased hydrophobicity of the PAO surface was used to favor the printing of ungerminated, hydrophobic[17] spores of *A. fumigatus* over cells of another fungus (*C. albicans*). As microorganisms vary in surface charge and hydrophobicity (both between strains, but also within the life cycle of some species – for example the surface of *A. fumigatus* spores become less hydrophobic as they germinate) this suggests interesting possibilities. The first is simply a level of enrichment and/or purification; then, as patterning of biomolecules and becomes more sophisticated, the scope may extend to very specific capture (organism, growth modality) based on tailored surface properties. Chemical modification of PAO to display functional antibodies, nucleic acids, peptides and carbohydrates is possible.[14,18] A second important modification possible on PAO is to subdivide the surface with physical barriers to allow printed cells to remain segregated after growth. Such high density arrays of microcolonies can be stained with fluorogenic dyes or other detection reagents, for example to reveal variants responding to changes in microbial metabolism, as part of a screening process to isolate improved industrial strains or identify pathogens. Other methods, such as Raman spectroscopy, are capable of assaying microcolonies *in situ* and determining the species.[19] Miniaturized sensors or downstream molecular analyses may be combined with dense arrays of microcolonies or single cells to create integrated MEMS devices.[20] Dense, printed arrays of microorganisms on PAO may also be a useful archival format. The techniques described here represent advances in microbial handling, high throughput screening and a contribution to creating more highly integrated "microbiology laboratories on a chip" and using printed microbes in the fabrication or functioning of MEMS devices.

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